PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :
C07H 15/12, C12P 21/00
C12N 15/00, C07K 13/00

A1

(11) International Publication Number: WO 87/ 02671

(43) International Publication Date: 7 May 1987 (07.05.87)

(21) International Application Number: PCT/US86/02269

(22) International Filing Date: 27 October 1986 (27.10.86)

(31) Priority Application Number: 793,980

(32) Priority Date: 1 November 1985 (01.11.85)

(33) Priority Country: US

(60) Parent Application or Grant
(63) Related by Continuation
US 793,980 (CIP)
Filed on 1 November 1985 (01.11.85)

(71) Applicant *(for all designated States except US)*: INTERNATIONAL GENETIC ENGINEERING, INC. [US/US]; 1545 - 17th Street, Santa Monica, CA 90404 (US).

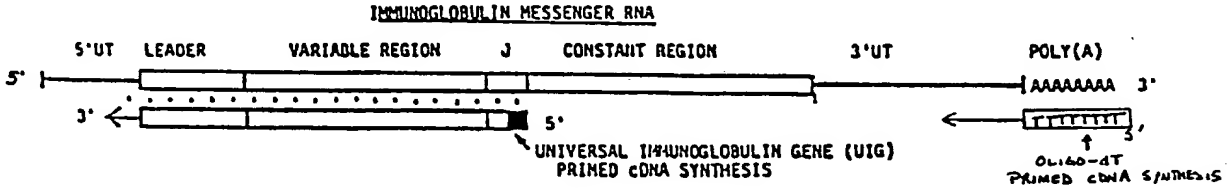
(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ROBINSON, Randy, R. [US/US]; 5606 W. 79th Street, Los Angeles, CA 90045 (US). LIU, Alvin, Y. [US/US]; 807 - 8th Street, Apt. 6, Santa Monica, CA 90403 (US). HORWITZ, Arnold, H. [US/US]; 7529 Midfield Avenue, Los Angeles, CA 90045 (US). WALL, Randolph [US/US]; 5106 Van Noord, Sherman Oaks, CA 91423 (US).

(74) Agents: GOLDSTEIN, Jorge, A. et al.; Saidman, Sterne, Kessler & Goldstein, 1225 Connecticut Ave., Suite 300, Washington, DC 20036 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU, US.

Published
With international search report.

(54) Title: MODULAR ASSEMBLY OF ANTIBODY GENES, ANTIBODIES PREPARED THEREBY AND USE



(57) Abstract

Chimeric immunoglobulin molecules are produced by cloning cDNA sequences encoding human constant regions together with non-human variable regions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TG	Togo
DK	Denmark	MC	Monaco	US	United States of America
FI	Finland	MG	Madagascar		

TITLE OF THE INVENTION

MODULAR ASSEMBLY OF ANTIBODY GENES, ANTIBODIES PREPARED THEREBY AND USE

BACKGROUND OF THE INVENTION

This application is a continuation in part of Application Serial No. 793,980, filed November 1, 1985, the contents of which are herein fully incorporated by reference.

Field of the Invention

This invention relates to recombinant DNA methods of preparing immunoglobulins, genetic sequences coding therefor, as well as methods of obtaining such sequences.

Background Art

The application of cell-to-cell fusion for the production of monoclonal antibodies by Kohler and Milstein (Nature (London), 256: 495, 1975) has spawned a revolution in biology equal in impact to the invention of recombinant DNA cloning. Hybridoma-produced monoclonal antibodies are already widely used in clin-

ical diagnoses and basic scientific studies. Applications of human B cell hybridoma-produced monoclonal antibodies hold great promise for the clinical treatment of cancer, viral and microbial infections, B cell immunodeficiencies with diminished antibody production, and other diseases and disorders of the immune system.

Unfortunately, yields of monoclonal antibodies from human hybridoma cell lines are relatively low (1 ug/ml in human x human compared to 100 ug/ml in mouse hybridomas), and production costs are high for antibodies made in large scale human tissue culture. Mouse x mouse hybridomas, on the other hand, are useful because they produce abundant amounts of protein, and these cell lines are more stable than the human lines. However, repeated injections of "foreign" antibodies, such as a mouse antibody, in humans, can lead to harmful hypersensitivity reactions.

There has therefore been recent exploration of the possibility of producing antibodies having the advantages of monoclonals from mouse-mouse hybridomas, yet the species specific properties of human monoclonal antibodies.

Another problem faced by immunologists is that most human monoclonal antibodies (i.e., antibodies having human recognition properties) obtained in cell culture are of the IgM type. When it is desirable to obtain human monoclonals of the IgG type, however, it has been necessary to use such techniques as cell sorting, to separate the few cells which have switched to producing antibodies of the IgG or other type from the majority producing antibodies of the IgM type. A

need therefore exists for a more ready method of switching antibody classes, for any given antibody of a predetermined or desired antigenic specificity.

The present invention bridges both the hybridoma and monoclonal antibody technologies and provides a quick and efficient method, as well as products derived therefrom, for the improved production of chimeric human/non-human antibodies, or of "class switched" antibodies.

INFORMATION DISCLOSURE STATEMENT*

Approaches to the problem of producing chimeric antibodies have been published by various authors.

Morrison, S. L. et al., Proc. Natl. Acad. Sci., USA, 81: 6851-6855 (November 1984), describe the production of a mouse-human antibody molecule of defined antigen binding specificity, produced by joining the variable region genes of a mouse antibody-producing myeloma cell line with known antigen binding specificity to human immunoglobulin constant region genes using recombinant DNA techniques. Chimeric genes were constructed, wherein the heavy chain variable region exon from the myeloma cell line S107 well joined to human IgG1 or IgG2 heavy chain constant region exons, and the light chain variable region exon from the same myeloma to the human kappa light chain exon. These genes were transfected into mouse myeloma cell lines

---

* Note: The present Information Disclosure Statement is subject to the provisions of 37 C.F.R. 1.97(b). In addition, Applicants reserve the right to demonstrate that their invention was made prior to any one or more of the mentioned publications.

and. Transformed cells producing chimeric mouse-human antiphosphocholine antibodies were thus developed.

Morrison, S. L. et al., European Patent Publication No. 173494 (published March 5, 1986), disclose chimeric "receptors" (e.g. antibodies) having variable regions derived from one species and constant regions derived from another. Mention is made of utilizing cDNA cloning to construct the genes, although no details of cDNA cloning or priming are shown. (see pp 5, 7 and 8).

Boulianne, G. L. et al., Nature, 312: 643 (December 13, 1984), also produced antibodies consisting of mouse variable regions joined to human constant regions. They constructed immunoglobulin genes in which the DNA segments encoding mouse variable regions specific for the hapten trinitrophenyl (TNP) were joined to segments encoding human mu and kappa constant regions. These chimeric genes were expressed as functional TNP binding chimeric IgM.

For a commentary on the work of Boulianne et al. and Morrison et al., see Munro, Nature, 312: 597 (December 13, 1984), Dickson, Genetic Engineering News, 5, No. 3 (March 1985), or Marx, Science, 229: 455 (August 1985).

Neuberger, M. S. et al., Nature, 314: 268 (March 25, 1985), also constructed a chimeric heavy chain immunoglobulin gene in which a DNA segment encoding a mouse variable region specific for the hapten 4-hydroxy-3-nitrophenacetyl (NP) was joined to a segment encoding the human epsilon region. When this chimeric gene was transfected into the J558L cell line, an antibody was produced which bound to the NP hapten and had human IgE properties.

Neuberger, M.S. et al., have also published work showing the preparation of cell lines that secrete hapten-specific antibodies in which the Fc portion has been replaced either with an active enzyme moiety (Williams, G. and Neuberger, M.S. Gene 43:319, 1986) or with a polypeptide displaying c-myc antigenic determinants (Nature, 312:604, 1984).

Neuberger, M. et al., PCT Publication WO 86/01533, (published March 13, 1986) also disclose production of chimeric antibodies (see p. 5) and suggests, among the technique's many uses the concept of "class switching" (see p. 6).

Taniguchi, M., in European Patent Publication No. 171 496 (published February 19, 1985) discloses the production of chimeric antibodies having variable regions with tumor specificty derived from experimental animals, and constant regions derived from human. The corresponding heavy and light chain genes are produced in the genomic form, and expressed in mammalian cells.

Takeda, S. et al., Nature, 314: 452 (April 4, 1985) have described a potential method for the construction of chimeric immunoglobulin genes which have intron sequences removed by the use of a retrovirus vector. However, an unexpected splice donor site caused the deletion of the V region leader sequence. Thus, this approach did not yield complete chimeric antibody molecules.

Cabilly, S. et al., Proc. Natl. Acad. Sci., USA, 81: 3273-3277 (June 1984), describe plasmids that direct the synthesis in E. coli of heavy chains and/or light chains of anti-carcinoembryonic antigen (CEA)

antibody. Another plasmid was constructed for expression of a truncated form of heavy chain (Fd') fragment in E. coli. Functional CEA-binding activity was obtained by in vitro reconstitution, in E. coli extracts, of a portion of the heavy chain with light chain.

Cabilly, S., et al., European Patent Publication 125023 (published November 14, 1984) describes chimeric immunoglobulin genes and their presumptive products as well as other modified forms. On pages 21, 28 and 33 it discusses cDNA cloning and priming.

Boss, M. A., European Patent Application 120694 (published October 3, 1984) shows expression in E. coli of non-chimeric immunoglobulin chains with 4-nitrophenyl specificity. There is a broad description of chimeric antibodies but no details (see p. 9).

Wood, C. R. et al., Nature, 314: 446 (April, 1985) describe plasmids that direct the synthesis of mouse anti-NP antibody proteins in yeast. Heavy chain mu antibody proteins appeared to be glycosylated in the yeast cells. When both heavy and light chains were synthesized in the same cell, some of the protein was assembled into functional antibody molecules, as detected by anti-NP binding activity in soluble protein prepared from yeast cells.

Alexander, A. et al., Proc. Nat. Acad. Sci. USA, 79: 3260-3264 (1982), describe the preparation of a cDNA sequence coding for an abnormally short human Ig gamma heavy chain (OMM gamma^3 HCD serum protein) containing a 19- amino acid leader followed by the first 15 residues of the V region. An extensive internal deletion removes the remainder of the V and the entire C_H1 domain. This is cDNA coding for an internally deleted molecule.

Dolby, T. W. et al., Proc. Natl. Acad. Sci., USA, 77: 6027-6031 (1980), describe the preparation of a cDNA sequence and recombinant plasmids containing the same coding for mu and kappa human immunoglobulin polypeptides. One of the recombinant DNA molecules contained codons for part of the CH_3 constant region domain and the entire 3' noncoding sequence.

Seno, M. et al., Nucleic Acids Research, 11: 719-726 (1983), describe the preparation of a cDNA sequence and recombinant plasmids containing the same coding for part of the variable region and all of the constant region of the human IgE heavy chain (epsilon chain).

Kurokawa, T. et al., ibid, 11: 3077-3085 (1983), show the construction, using cDNA, of three expression plasmids coding for the constant portion of the human IgE heavy chain.

Liu, F. T. et al., Proc. Nat. Acad. Sci., USA, 81: 5369-5373 (September 1984), describe the preparation of a cDNA sequence and recombinant plasmids containing the same encoding about two-thirds of the CH_2, and all of the C_H3 and C_H4 domains of human IgE heavy chain.

Tsujimoto, Y. et al., Nucleic Acids Res., 12: 8407-8414 (November 1984), describe the preparation of a human V lambda cDNA sequence from an Ig lambda-producing human Burkitt lymphoma cell line, by taking advantage of a cloned constant region gene as a primer for cDNA synthesis.

Murphy, J., PCT Publication WO 83/03971 (published November 24, 1983) discloses hybrid proteins made of fragments comprising a toxin and a cell-specific ligand (which is suggested as possibly being an antibody).

Tan, et al., J. Immunol. 135:8564 (November, 1985), obtained expression of a chimeric human-mouse immunoglobulin genomic gene after transfection into mouse myeloma cells.

Jones, P. T., et al., Nature 321:552 (May 1986) constructed and expressed a genomic construct where CDR domains of variable regions from a mouse monoclonal antibody were used to substitute for the corresponding domains in a human antibody.

Sun, L.K., et al., Hybridoma 5 suppl. 1 S17 (1986), describes a chimeric human/mouse antibody with potential tumor specificty. The chimeric heavy and light chain genes are genomic constructs and expressed in mammalian cells.

Sahagan et al., J. Immun. 137:1066-1074 (August 1986) describe a chimeric antibody with specificity to a human tumor associated antigen, the genes for which are assembled from genomic sequences.

For a recent review of the field see also Morrison, S.L., Science 229: 1202-1207 (September 20, 1985) and Oi, V. T., et al., BioTechniques 4:214 (1986).

The Oi, et al., paper is relevant as it argues that the production of chimeric antibodies from cDNA constructs in yeast and/or bacteria is not necessarily advantageous.

See also Commentary on page 835 in Biotechnology 4 (1986).

SUMMARY OF THE INVENTION

The invention provides a novel approach for producing genetically engineered antibodies of desired variable region specificity and constant region properties through gene cloning and expression of light and heavy chains. The cloned immunoglobulin gene products can be produced by expression in genetically engineered organisms.

The application of chemical gene synthesis, recombinant DNA cloning, and production of specific immunoglobulin chains in various organisms provides an effective solution for the efficient large scale production of human monoclonal antibodies with the antigen specificities of either human or non-human, especially rodent, monoclonal antibodies. The invention also provides a solution to the problem of class switching antibody molecules, so as to readily prepare immunoglobulins of a certain binding specificity of any given class.

The invention provides cDNA sequences coding for immunoglobulin chains comprising a constant human region and a variable, either human or non-human, region. The immunoglobulin chains can either be heavy or light.

The invention also provides gene sequences coding for immunoglobulin chains comprising a cDNA variable region of either human or non-human origin and a genomic constant region of human origin.

The invention also provides sequences as above, present in recombinant DNA molecules, especially in vehicles such as plasmid vectors, capable of expression in desired prokaryotic or eukaryotic hosts.

The invention also provides consensus sequences and specific oligonucleotide sequences useful as probes for hybridization and priming cDNA synthesis of any hybridoma mRNA coding for variable regions of any desired specificity.

The invention provides hosts capable of producing, by culture, chimeric antibodies and methods of using these hosts.

The invention also provides chimeric immunoglobulin individual chains and whole assembled molecules having human constant regions and non-human variable regions, wherein both variable regions have the same binding specificity.

Among other immunoglobulin chains and/or molecules provided by the invention are:

(a) a complete functional, immunoglobulin molecule comprising:
  (i) two identical chimeric heavy chains comprising a non-human variable region and human constant region and
  (ii) two identical all (i.e. non-chimeric) human light chains.

(b) a complete, functional, immunoglobulin molecule comprising:
  (i) two identical chimeric heavy chains comprising a non-human variable region and a human constant region, and
  (ii) two identical all (i.e. non-chimeric) non-human light chains.

(c) a monovalent antibody, i.e., a complete, functional immunoglobulin molecule comprising:
  (i) two identical chimeric heavy chains comprising a non-human variable region and a human constant region, and
  (ii) two different light chains, only one of which has the same specificity as the variable region of the heavy chains. The resulting antibody molecule binds only to one end thereof and is therefore incapable of divalent binding;

(d) an antibody with two different specificities, i.e., a complete, functional immunoglobulin molecule comprising:

(i) two different chimeric heavy chains, the first one of which comprises a non-human variable region and a human constant region and the second comprises a different non-human variable region, and a human constant region, and

(ii) two different chimeric light chains, the first one of which comprises a non-human variable region having the same specificity as the first heavy chain variable region, and a human constant region, and the second comprises a non-human variable region having the same specificity as the second heavy chain variable region, and a human constant region.

The resulting antibody molecule binds to two different antigens.

Genetic sequences, especially cDNA sequences, coding for the aforementioned combinations of chimeric chains or of non-chimeric chains are also provided herein.

The invention also provides for a genetic sequence, especially a cDNA sequence, coding for the variable region of an antibody molecule heavy and/or light chain, operably linked to a sequence coding for a polypeptide different than an immunoglobulin chain (e.g., an enzyme). These sequences can be assembled by the methods of the invention, and expressed to yield mixed-function molecules.

The use of cDNA sequences is particularly advantageous over genomic sequences (which contain introns), in that cDNA sequences can be expressed in bacteria or other hosts which lack RNA splicing systems.

Among preferred specific antibodies are those having specificities to cancer-related antigens.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows the DNA rearrangements and the expression of immunoglobulin mu and gamma heavy chain genes. This is a schematic representation of the human heavy chain gene complex, not shown to scale. Heavy chain variable V region formation occurs through the joining of V_H, D and J_H gene segments. This generates an active mu gene. A different kind of DNA rearrangement called "class switching" relocates the joined V_H, D and J_H region from the mu constant C region to another heavy chain C region (switching to gamma is diagrammed here). The scheme emphasizes that the J region is a common feature of all expressed heavy chain genes. The J region is also a common feature of expressed light chain genes.

FIGURE 2 shows the known nucleotide sequences of human and mouse J regions. Consensus sequences for the J regions are shown below the actual sequences. The oligonucleotide sequence below the mouse kappa J region consensus sequence is a Universal Immunoglobulin Gene (UIG) oligonucleotide which is used in the present invention.

FIGURE 3 shows a scheme noting the use of the UIG oligonucleotide primer for the synthesis of cDNA complementary to the variable region of immunoglobulin messenger RNA, or the use of oligo-dT as a primer for cDNA synthesis, followed by *in vitro* mutagenesis.

FIGURE 4 shows the synthesis and analysis of human IgG1 genes, including three isolated clones (A.b), one of which (pGMH-6) is utilized as a cloning vector (B). A 1.5 kb deletion of pBR322 sequence between *Bam* HI and *Pvu*II is marked. Not to scale.

FIGURE 5 shows the cloning vector pQ23, a modified pBR322, useful for cDNA cloning at the KpnI site. This vector also contains the useful restriction enzyme sites *Bgl*II plus *Sal*I. Not to scale.

FIGURE 6 shows in A. the synthesis and analysis of human light chain *kappa* genes. The Figure also shows in B. (not to scale) construction of a human C_K region cloning vector pING2001.

FIGURE 7 shows primers designed for immunoglobulin V region synthesis. (A) shows the heavy chain J-C regions and primers. A DNA version of each mouse J heavy region is shown directly above primers designed from that sequence. Mouse J regions are 5' to 3', left to right, while primers are 3' to 5', left to right. Primer names are included in brackets, and numbers of nucleotides (N) and number of mismatches with each J_H region are listed to the right. Primers which introduce a *Bst*EII site are underlined. (B) shows the light chain J regions and primers. The same as for (A) except for light chains. Primers designed to introduce a *Bgl*II site are underlined, as is the *Bcl*I site present in pING2016E. (C) shows mouse vari-

able region consensus UIG primers. The actual primer sequence is shown below that consensus sequence. The human C_K HindIII vector pGML60 is shown below. (D) shows a mouse gamma 2a J/C junction primer.

FIGURE 8 shows the synthesis of heavy chain V region module genes using oligonucleotide primed cDNA synthesis. Not to scale.

FIGURE 9 shows the construction of hybrid mouse-human immunoglobulin genes. Panel A shows construction of a heavy chain gene. Stippled regions show C region modules, while hatched or black regions show V region modules. Not to scale.

FIGURE 10 shows the construction of cDNA cloning-expression shuttle vectors for mammalian cells. The vectors pING2003 and pING2003E are derived from pL1, pUC12, pSV2-neo and M8-alphaRX12. Stippled regions indicate mouse heavy chain enhancer DNA, hatched regions indicate SV-40 DNA from pL1, and cross-hatched regions indicate SV-40 DNA from pSV2-neo. In the vectors pING2003 and pING2003E, thick lines represent pBR322 DNA from pSV2-neo, while thin lines represent pUC12 DNA. Arrows indicate the locations and directions of SV-40 early region promoters, and indicates a complete SV-40 intron sequence. Not to scale.

FIGURE 11 shows the construction of the heavy chain expression plasmid pING2006E. Arrows show SV-40 promoter locations and directions of transcription. Hatched and black areas show mouse V region modules, while stippled areas show human C region modules. Not to scale.